

Procedures for Familial Comparison and Interpretation Using the KIn CALc Kinship Software (v. 5.0.10_FBI)

1 Scope

These procedures describe the methods by which DNA typing results are interpreted for familial comparison purposes using kinship analysis software, KIn CALc.

2 Background

Upon completion of the technical aspects of DNA analysis, the DNA typing results must be first verified and interpreted by an Examiner using the methods established in the appropriate interpretation standard operating procedure (SOP) of the *DNA Procedures Manual*.

Based on the transmission of genetic material within a family, DNA typing results can potentially be used to establish the likelihood of biological relatedness. DNA typing results obtained from potential relatives (e.g., Unidentified Human Remains [UHRs] and relatives of a missing person, an alleged parent and a child) may be compared and used in statistical kinship assessments using the kinship analysis software KIn CALc. Based on manual comparisons of the DNA typing results (e.g., autosomal STR, Y-STR, mitochondrial DNA testing), an Examiner may be able to determine whether an individual can be excluded as a potential biological relative, and a kinship calculation is not necessary.

KIn CALc is an Excel-based program that allows the user to evaluate a putative familial relationship, given the DNA typing results of a “Test” sample and other “Reference” sample(s). The Test sample may be a sample from evidence (e.g., a UHR sample) or a known reference sample for which the relationship to the other known reference samples is in question (e.g., paternity analysis). The software is used to calculate a likelihood ratio (LR) or combined kinship index (KI) from multiple population databases. The KI conveys the ratio of the probabilities of observing the DNA profiles under two mutually exclusive hypotheses: (1) that the Test and Reference(s) are biologically related in the manner assessed and (2) generally that the Test and Reference(s) are unrelated. Generally a KI greater than one supports the hypothesis of relatedness and a KI less than one supports the alternate hypothesis, generally, of unrelatedness. The pedigree assessment is based on the information provided by the contributor. Requests for additional permutations of the pedigree must be approved by the Technical Leader (TL). These conclusions are compiled by the Examiner into a written report and are the official FBI Laboratory findings as to the nuclear DNA typing results.

3 Equipment/Materials/Reagents

KIn CALc Software Version 5.0.10_FBI

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4 Analytical Procedures

When a manual comparison of the results excludes the potential relationship (see section 5.1.4), use of the KIn CALc software is not necessary.

4.1 Software-assisted Kinship Calculations

4.1.1 Determination of Hypotheses

The primary hypothesis is the probability of observing the DNA results if the Reference(s) is (are) biologically related to the Test. The alternate hypothesis is generally the probability of observing the DNA results if the Reference sample(s) is (are) unrelated to the Test. The Examiner will determine the type of relationship to be assessed based on the case information (e.g., the Reference samples are from the parents of a missing person, and the Test is from a UHR which is potentially that missing person).

4.1.2 Kinship Calculation using KIn CALc

4.1.2.1 Theta

The theta value is by default set to 0.01 for kinship analysis using KIn CALc. This value should be used when assessing the relationship likelihoods in African American, Caucasian, Southwestern Hispanic, Southeastern Hispanic, Filipino, Trinidad or Chamorro populations. Theta should be manually changed to 0.03 when determining the likelihood of a relationship in Native American populations (Apache, Navajo or Minnesota).

4.1.2.2 Linkage

4.1.2.2.1 Two pairs of loci are linked closely enough to affect the KI in some situations:¹

- CSF1P0 and D5S818
- D12S391 and vWA

4.1.2.2.2 For simple paternity cases (i.e., a paternity duo or trio where the TEST is the alleged parent) and for simple reverse paternity cases (i.e., a reverse paternity duo or trio where the TEST is the alleged child), all loci may be used in the KI calculation if results are available.

4.1.2.2.3 Both loci of a linked pair must not be used in the KI calculation for pedigrees that are not simple paternity or simple reverse paternity.² The locus used in the calculations should be the more discriminating of the pair. If results are only available for the less discriminating locus,

¹ Despite their location, there is no evidence of linkage disequilibrium for the aforementioned markers at the population level due to, presumably, the re-assortment of alleles throughout sufficient generations. Therefore, these markers can be considered 'independent' for calculations involving unrelated individuals.

² There are additional specific situations in which it would be appropriate to use both loci of a linked pair. To avoid these complexities, a more conservative approach is used for the purposes of this SOP: only one locus of a linked pair will be used in KI calculations unless the assessment is for paternity or reverse paternity.

it may be used in the KI calculation.

- CSF1P0 is more discriminating than D5S818
- D12S391 is more discriminating than vWA

4.1.2.3 Input of DNA Profile Information

GlobalFiler™ (GF) profiles must be analyzed with AT150 to be assessed using the KIn CALc software, i.e., Globalfiler AT50 profiles cannot be evaluated with the KIn CALc software. Identifiler® Plus (ID+) profiles must be analyzed at AT50 to be assessed with the KIn CALc software. Any locus with only one allele with a peak height less than the respective stochastic threshold (ST) (i.e., 725 RFU for GF and 200 RFU for ID+) must not be entered into KIn CALc. These loci are inconclusive for KI calculations. For single source profiles, if a locus has two alleles, both alleles may be entered into KIn CALc regardless of peak height.

4.1.2.3.1 Option 1: Manual Entry

4.1.2.3.1.1 Open the KIn CALc software and navigate to the “Kit Conversion” tab.

4.1.2.3.1.2 Enter the sample identifier for the “Test” sample (e.g., item 5) under the cell labeled “Item #” in the “Commercial Multiplex Format” column (yellow column). For paternity cases the “Test” will always be the alleged parent. Enter the DNA profile results obtained for the Test.³ **In the KinCalc software, the alleles must always be entered in increasing numerical order for each locus (e.g., 11, 12 not 12, 11). Additionally, if a locus is homozygous, the allele must be entered into both the “Allele 1” and “Allele 2” rows.** Once all genetic data is entered, select the “Insert Test Profile” button. See Figure 1.

4.1.2.3.1.3 Enter the sample identifier for the first Reference under the cell labeled “Item #” and enter the DNA profile results obtained for the Reference in the cells corresponding to the loci for which data is available.⁴ Once complete, click the “Insert AR1 Profile” button.

4.1.2.3.1.4 If multiple reference samples are available, repeat step 4.1.2.3.1.3, selecting the appropriate “Insert AR# Profile” button, until the information for all samples has been entered.

4.1.2.3.1.5 Once all references are entered, click the “To Pedigree” or “To Custom Pedigree” button as appropriate or manually navigate to the appropriate tab.

³Only alleles that comply with the procedures set for use in statistical analysis will be entered. Refer to the appropriate interpretation SOP of the *DNA Procedures Manual*. The sex typing results from the amelogenin locus are not included in kinship index calculations.

⁴ Not all References will add value to the calculation. For example, if both parents are available, adding a sibling to the calculation does not change the final result. Likewise, if the father is available, the paternal grandparents are not needed. Only the References that contain additional genetic information not otherwise represented need to be used in the calculation.

KIT CONVERSION WORKSHEET

- 1) Using the drop-down menus, arrange the loci in the order of the commercial multiplex employed.
- 2) Click on the respective button to insert the Kin CALc conversion into the Profiles worksheet.

Kin CALc 5.0.10 FBI

Commercial Multiplex			Kin CALc		
	Format	Item #		Format	Item #
D3S1358	Allele 1		CSF1PO	Allele 1	
	Allele 2			Allele 2	
vWA	Allele 1		D10S1248	Allele 1	
	Allele 2			Allele 2	
D16S539	Allele 1		D12S391	Allele 1	
	Allele 2			Allele 2	
CSF1PO	Allele 1		D13S317	Allele 1	
	Allele 2			Allele 2	
TPOX	Allele 1		D16S539	Allele 1	
	Allele 2			Allele 2	
D8S1179	Allele 1		D18S51	Allele 1	
	Allele 2			Allele 2	
D21S11	Allele 1		D19S433	Allele 1	
	Allele 2			Allele 2	
D18S51	Allele 1		D1S1656	Allele 1	
	Allele 2			Allele 2	
D2S441	Allele 1		D21S11	Allele 1	
	Allele 2			Allele 2	
D19S433	Allele 1		D22S1045	Allele 1	
	Allele 2			Allele 2	
TH01	Allele 1		D2S1338	Allele 1	
	Allele 2			Allele 2	
FGA	Allele 1		D2S441	Allele 1	
	Allele 2			Allele 2	
D22S1045	Allele 1		D3S1358	Allele 1	
	Allele 2			Allele 2	
D5S818	Allele 1		D5S818	Allele 1	
	Allele 2			Allele 2	
D13S317	Allele 1		D6S1043	Allele 1	
	Allele 2			Allele 2	
D7S820	Allele 1		D7S820	Allele 1	
	Allele 2			Allele 2	
SE33	Allele 1		D8S1179	Allele 1	
	Allele 2			Allele 2	
D10S1248	Allele 1		F13A01	Allele 1	
	Allele 2			Allele 2	
D1S1656	Allele 1		F13B	Allele 1	
	Allele 2			Allele 2	
D12S391	Allele 1		FESFPS	Allele 1	
	Allele 2			Allele 2	
D2S1338	Allele 1		FGA	Allele 1	
	Allele 2			Allele 2	

Insert Test Profile

Insert AR1 Profile

Insert AR2 Profile

Insert AR3 Profile

Insert AR4 Profile

Insert AR5 Profile

Insert AR6 Profile

Insert AR7 Profile

Insert AR8 Profile

Insert AR9 Profile

Insert AR10 Profile

Clear Data

To Profiles

To Intro

To Pedigree

To Custom Pedigree

To KI

Figure 1 – Entering profiles in the “Kit Conversion” tab

4.1.2.3.2 Option 2: Import txt file from GeneMapper® ID-X (GMIDX)

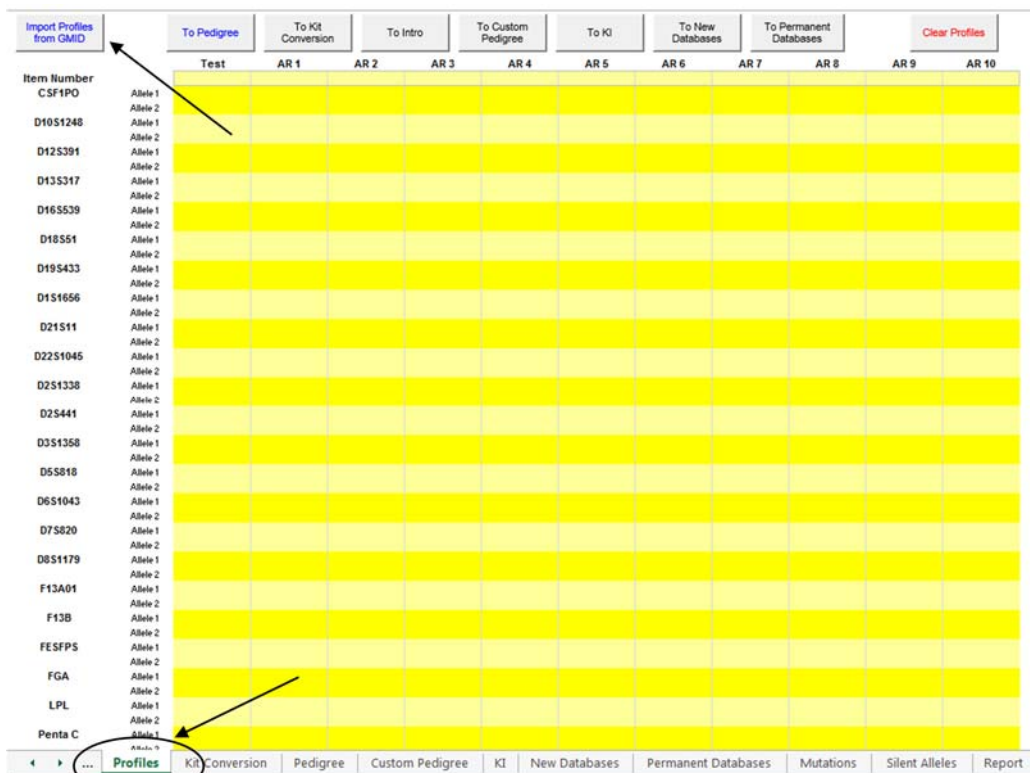
4.1.2.3.2.1 In GeneMapper® ID-X (GMIDX), highlight the sample(s) to be imported into KIn CALc and click on the “Display Plots” icon.

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4.1.2.3.2.2 From the plots, select the “Sizing Table” icon. Go to “File” – “Export Table”. Save the generated .txt file.

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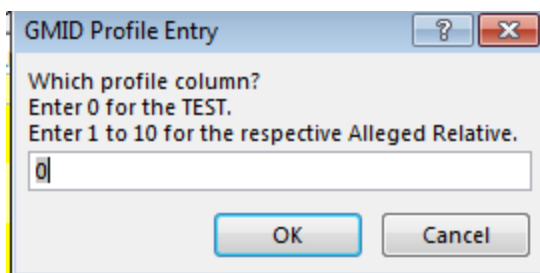
4.1.2.3.2.3 Open the KIn CALc software and navigate to the “Profiles” tab. Click on the “Import Profiles from GMID” button located on the upper left hand corner of the screen.



4.1.2.3.2.4 Locate exported table and click “Open”. From the “Import Profile from an Exported Table” box, select the profile (one at a time) to be imported into the software and click “OK”. Ensure that the displayed sample name is correct and click “OK”. If the sample name is incorrect, the user can make the required changes in the appropriate field before continuing to the next step.

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enter sequential numbers from “1-10” for each of the samples until all of the profiles have been entered.



4.1.2.3.2.6 Repeat the previous steps until all samples needed to establish the pedigree have been entered into the software tool.

4.1.2.3.2.7 Once all references are entered, click the “To Pedigree” or “To Custom Pedigree” button as appropriate or manually navigate to the appropriate tab.

4.1.2.4 Establishing Alleged Relationship(s) on the Pedigree

Navigate to the “Pedigree” or “Custom Pedigree” tab at the bottom of the screen, as appropriate. The custom pedigree tab should be used in situations that cannot be evaluated using the standard pedigree tab (see 4.1.2.4.2).

4.1.2.4.1 Pedigree Tab for Standard Pedigrees

4.1.2.4.1.1 In the pedigree screen, the Test will always be pre-selected. Select the box(es) corresponding to the reference sample(s) relationship(s) to the Test. For example, if item 1 is the alleged mother of the Test, select the box in the pedigree that corresponds to the mother. If item 2 is the alleged full-sibling of the Test, select a box in the pedigree that corresponds to a full-sibling. See Figure 2. For paternity cases, the alleged parent will always be the ‘Test’ and the known parent and child will be assigned the boxes corresponding to ‘Test Mate’ and ‘Test-Test Mate-Child’, respectively. See Figure 3.

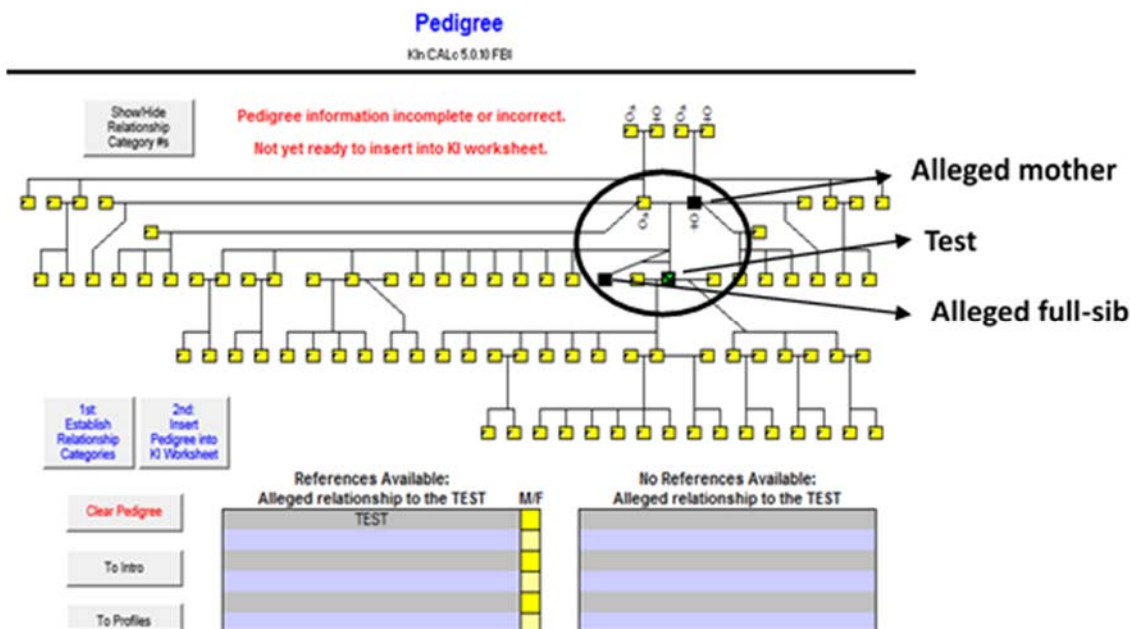


Figure 2 – The “Pedigree” tab

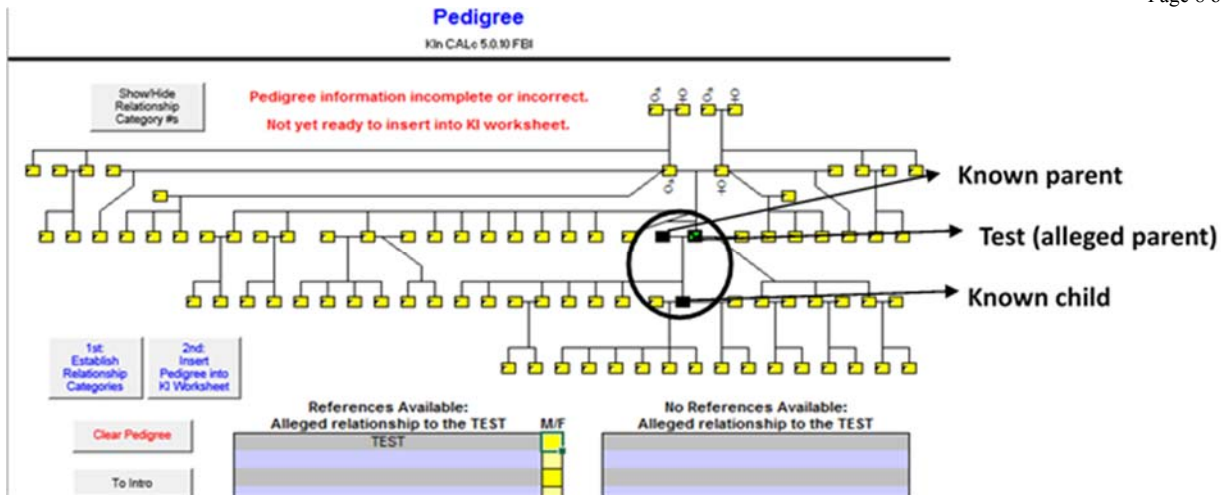


Figure 3 – The “Pedigree” tab for Paternity Analysis

4.1.2.4.1.2 Click on the “1st: Establish Relationship Categories” button on the left side of the screen. This action will populate the “References Available” box with the relatives selected in the pedigree. The “No References Available” Box will be auto-populated with those relatives for which information is not entered but whose information would have been required to establish the genotypes of the available individuals. For example, to correctly infer the genotype of the Test and full-sibling, both parents’ genotypes are required. If the genotype for the father is not entered, the software will generate his potential genotypes in order to perform the calculation; therefore, “father” will be auto-populated in the “No References Available box.” See Figure 4.

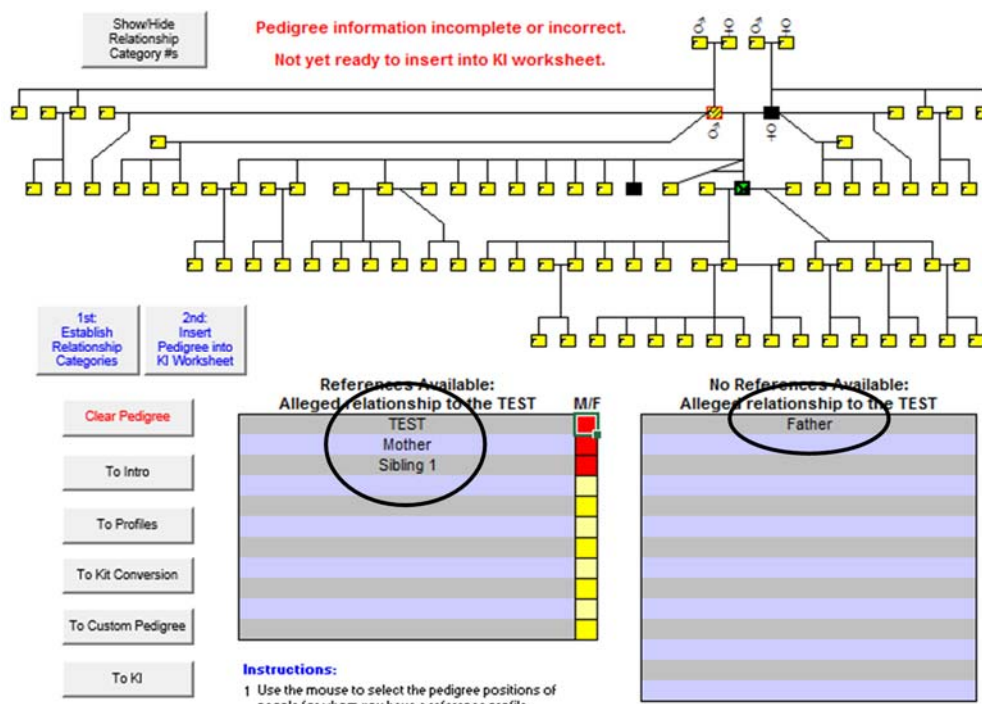


Figure 4 – Establishing Relationship Categories in the “Pedigree” tab

4.1.2.4.1.3 Verify that the relationships described in the References available box correspond to the actual References available. If not, go back to the pedigree and deselect the incorrect box in the pedigree, then select the correct pedigree box and click on the “1st: Establish Relationship Categories” button again. The “References Available” and “No References Available” boxes will be updated with the new information.

4.1.2.4.1.4 Enter the gender of the Test and References by typing the letter “M” for male and “F” for female in the red box next to the relative listed in the “Reference available” box. The boxes will change to yellow once the information is entered and will all turn green once the gender for all samples are entered. The message in red at the top of the screen will change to green to indicate that all the information needed to perform the kinship index calculation has been completed and the user can proceed to the calculation screen. See Figure 5.

The screenshot displays the 'Pedigree' tab interface. At the top, a message box states 'Pedigree complete. Ready to insert into KI worksheet.' in green. Below this is a pedigree chart with yellow boxes representing individuals. To the left of the chart are two buttons: '1st: Establish Relationship Categories' and '2nd: Insert Pedigree into KI Worksheet'. Below these are several navigation buttons: 'Clear Pedigree', 'To Intro', 'To Profiles', 'To Kit Conversion', 'To Custom Pedigree', 'To KI', 'To New DBs', and 'To Permanent DBs'. To the right of the navigation buttons are two boxes: 'References Available: Alleged relationship to the TEST' and 'No References Available: Alleged relationship to the TEST'. The 'References Available' box contains a list of relationships: 'TEST', 'Mother', 'Sibling 1', and several empty rows. Each row has a red box next to it for entering gender. The 'TEST' row has a green box with an 'M' (male) entered. The 'Mother' row has a green box with an 'F' (female) entered. The 'Sibling 1' row has a green box with an 'F' (female) entered. The 'No References Available' box contains a list of relationships: 'Father' and several empty rows. Each row has a red box next to it for entering gender. Below the boxes are 'Instructions' and a 'Note'.

Instructions:

- 1 Use the mouse to select the pedigree positions of people for whom you have a reference profile.
- 2 Click on "Establish Relationship Categories".
- 3 Enter the known gender of the people with references, regardless of whether or not there are gender identifying DNA results.
- 4 Click on "Insert Pedigree into KI Worksheet".

Note:

- The green box with an X in it is the position of the TEST person (e.g., the possible remains of a missing person, or the alleged father in a paternity.) This is the person who is in question, and whose inclusion in the pedigree

Figure 5 – Entering gender in the “Pedigree” tab

4.1.2.4.2 Custom Pedigree Tab for Non-Standard Pedigrees

Drawing the pedigree(s) can aid in the use of the custom pedigree function. The custom pedigree tab may be used in two situations. Use of the custom pedigree tab to assess any scenario besides the two described below may only be conducted with TL approval.

(A) To assess scenarios for which:

- mutations must be allowed for the pedigree to be true,
- the No References Available box is populated, and

- relationships other than parents or grandparents are populated in the “No References Available” box because mutation rates are gender specific.

In this scenario, the alternative hypothesis is unrelatedness. Go to the Custom Pedigree tab and set the “Manual alternate pedigree?” to “NO”, which prompts the software to automatically calculate the default denominator (i.e., unrelatedness). Enter the appropriate information in the “References Available” and “References Not Available” boxes, only using the “Primary Pedigree” area. Assign numbers 1-10 to samples for which genetic information is available, and 20-33 for any samples without genetic information but which are necessary to assess the relationship(s) in question. The “Test” sample will always be represented by number 17.

(B) To assess scenarios for which the alternate hypothesis is **not** unrelatedness: Go to the Custom Pedigree tab and set the “Manual Alternate Pedigree?” to “YES”, which allows for the alternate hypothesis to be defined. Using the “References Available” and “References Not Available” boxes, fill out the information corresponding to the Numerator in the “Primary Pedigree” area and the Denominator in the “Manual/Alternate Pedigree” area. Assign numbers 1-10 to samples for which genetic information is available, and 20-33 for any samples without genetic information but which are necessary to assess the relationship(s) in question. The “Test” sample will always be represented by number 17. The Primary Pedigree and Manual Alternate Pedigrees are independent; therefore, an individual represented by “1” in the Primary Pedigree does not need to be “1” in the Manual Alternate Pedigree. **When entering the information in the References boxes, always start from the most distant relatives available.**

- For example, are items 1 and 2 more likely from $\frac{3}{4}$ siblings (the fathers of the alleged siblings are full brothers, and the alleged siblings have the same mother) or from $\frac{1}{2}$ siblings (the fathers of the alleged siblings are unrelated, and the alleged siblings have the same mother)? Figure 7 shows the pedigrees generated for each of the two hypotheses. Figure 8 shows the custom pedigree tab for this example.

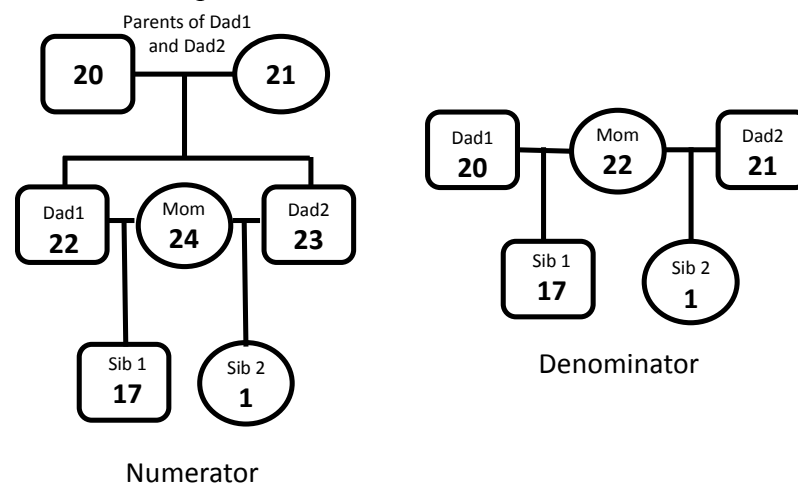


Figure 7 – Numerator and Denominator Hypothesis Pedigrees

CUSTOM PEDIGREES Kin CALc 5.0.10 FBI [See below for instructions.](#)

Pedigrees that will not fit into the structure found on the Pedigree worksheet may be entered here.
Note: The report for KIs calculated using a Custom Pedigree will not have a graphical component.

Manual alternate pedigree? ☒ **Yes** OK to transfer to KI? ☒ **TRUE**

Note: Entries in the second pedigree will not be used unless "Yes" is selected.

Monozygotic twin category:

Note: Monozygotic twin must be the last reference entered. Do not enter parents for this person.

[Insert Pedigree\(s\) into KI Worksheet](#)

References Available

Pedigree Member's Category	Gender (M/F)	Biological Father's Category	Biological Mother's Category
17	M	22	24
1	F	23	24
2			
3			
4			
5			
6			
7			
8			
9			
10			

Note: Do not skip rows when entering the pedigree(s).

References Not Available

Pedigree Member's Category	Gender (M/F)	Biological Father's Category	Biological Mother's Category
20	M		
21	F		
22	M	20	21
23	M	20	21
24	F		
25			
26			
27			
28			
29			
30			
31			
32			
33			

References Available

Pedigree Member's Category	Gender (M/F)	Biological Father's Category	Biological Mother's Category
17	M	20	22
1	F	21	22
2			
3			
4			
5			
6			
7			
8			
9			
10			

References Not Available

Pedigree Member's Category	Gender (M/F)	Biological Father's Category	Biological Mother's Category
20	M		
21	M		
22	F		
23			
24			
25			
26			
27			
28			
29			
30			
31			
32			
33			

Figure 8 – Custom Pedigree Tab

4.1.2.4.3 Performing the Kinship Index (KI) (or Paternity Index [PI]) Calculation

4.1.2.4.3.1 From either the Pedigree tab or the Custom Pedigree tab, click “2nd: Insert Pedigree into KI Worksheet.” This action will take the user to the calculator screen on the “KI” tab.

4.1.2.4.3.2 Go to the “Item #” column and select the appropriate sample identifier label from the drop-down menu for each of the samples. As the sample identifiers are selected, the software will populate loci and allele columns with the DNA data entered by the user in the “Profile ID” tab. See Figure 9.

The screenshot shows the 'KI' (Kinship Index) tab in a software application. At the top, there is a pedigree chart with a grid of cells representing relationships between individuals. Below the pedigree, there is a table with columns for 'Num', 'Den', 'KI', and 'Onset'. The table contains data for various sample identifiers, including CYP19D, D1S10, D1S11, D1S12, D1S13, D1S14, D1S15, D1S16, D1S17, D1S18, D1S19, D1S20, D1S21, D1S22, D1S23, D1S24, D1S25, D1S26, D1S27, D1S28, D1S29, D1S30, D1S31, D1S32, D1S33, D1S34, D1S35, D1S36, D1S37, D1S38, D1S39, D1S40, D1S41, D1S42, D1S43, D1S44, D1S45, D1S46, D1S47, D1S48, D1S49, D1S50, D1S51, D1S52, D1S53, D1S54, D1S55, D1S56, D1S57, D1S58, D1S59, D1S60, D1S61, D1S62, D1S63, D1S64, D1S65, D1S66, D1S67, D1S68, D1S69, D1S70, D1S71, D1S72, D1S73, D1S74, D1S75, D1S76, D1S77, D1S78, D1S79, D1S80, D1S81, D1S82, D1S83, D1S84, D1S85, D1S86, D1S87, D1S88, D1S89, D1S90, D1S91, D1S92, D1S93, D1S94, D1S95, D1S96, D1S97, D1S98, D1S99, D1S100. The table also includes columns for 'Report this?' and 'Report this?'. On the right side, there is a 'Progress Markers' section with a table showing the progress of calculations for different markers. A red circle highlights the 'Relationship to the test person' dropdown menu in the top left corner.

Figure 9 – Associating sample identifiers with the pedigree in the “KI” tab

4.1.2.4.3.3 From the drop-down menus at the bottom of the screen, the user must select the population frequency databases with which calculations will be performed. Kinship indices are calculated using four United States population groups (i.e., African-American, Caucasian, Southwestern Hispanic, and Southeastern Hispanic). Additional kinship indices are calculated when samples potentially originate from Native American populations (i.e., Apache, Navajo, and Minnesota Native American), or Caribbean populations (i.e., Trinidadian).^{5,6} See Figure 10. Note that selecting a “blank” database (e.g., when Southeastern Hispanic is calculated) will default the column to a previously used database. Select “No” for “Report this?” under the combined KI so that the value is not included on the report. The maximum number of populations per KIn CALc report is three. Generally, the African-American, Caucasian, and Southwestern Hispanic databases are selected first.

⁵ The use of the Native American or Caribbean population databases is generally based on the geographic location of the requesting agency or the population with which the known references in the pedigree associate themselves. With TL approval, other population databases may be similarly used.

⁶ The allele frequency distributions for the African American, Caucasian, Southeastern Hispanic, Southwestern Hispanic, Apache, Navajo, Trinidadian, Chamorro, and Filipino populations are published in: Moretti TR, Moreno LI, Smerick JB, Pignone ML, Hizon R, Buckleton JS, Bright J-A, Onorato AJ. Population data on the expanded CODIS core STR loci for eleven populations of significance for forensic DNA analyses in the United States. *Forensic Science International: Genetics* (2016) 25: 175-181. The allele frequency distributions for the Minnesota Native American population are found in the appropriate *DNA Procedures Manual*. Other sources of allele frequency distributions must be approved by the TL.

The screenshot displays the 'KI' (Kinship Index) tab in the DNA software. On the left, there is a pedigree chart showing relationships between individuals. The central area contains a table with columns for various loci (e.g., D1S1, D2S1, D3S1, etc.) and rows for different population databases. The 'Select Databases' button is circled in red. On the right, there are several panels for settings, including 'PROBABILITY SETTINGS' and 'CALCULATION SETTINGS'. The 'Change Mutation Approach' button is also visible.

Figure 10 – Selecting population databases in the “KI” tab

4.1.2.4.3.4 By default, the “Mutations” box is set to “0,” which means that the software will not allow for mutations in the calculation. Based on comparisons of DNA typing results of the Test and Reference samples, if inconsistencies in the typing results indicate that a mutation might have occurred (at three loci or less), the Examiner should click on the “Change Mutation Approach” button on the lower right side of the screen and select “1” for the mutation approach.^{7,8}

4.1.2.4.3.5 The gender of all samples in the pedigree must be defined for calculations that include mutations.⁹ If a mutation is necessary for the pedigree to be true, the examiner should ensure that the No References Available list in the Pedigree tab are only those with pre-determined genders in the pedigree (i.e., parents or grandparents of the TEST). If they are not, the pedigree should be constructed in the custom pedigree tab so that the genders of all individuals for both the References Available and No References Available can be defined before calculating the KI.

4.1.2.4.3.6 Click on the “KI” button; this action will populate the chosen population columns with the values corresponding to the numerator, denominator and likelihood ratio result for each of the loci for which information was entered. It will also compute the “Combined KI” (product of individual locus KIs) for each of the populations. If the kinship index is zero, the pedigree cannot be true without allowing mutations. The locus(i) requiring a mutation allowance can be identified by looking at the “Num” (Numerator) and “Den” (Denominator) column for each locus. Any locus with a value of “0” means mutations must be allowed for the pedigree to be true. Refer to 4.1.2.4.3.4 to change the mutation approach.

⁷ This approach was described by Ayres (2000) and is applied to all loci in the profile regardless of which locus requires the allowance for mutation.

⁸ When the DNA typing results of one of multiple relatives is not consistent with the pedigree in the manner described by the contributor, the Examiner may perform two calculations: one with all of the relatives, and one with the relative that does not fit removed. Both KI and CALc reports will be maintained, and the Examiner may report the results with fewer relatives.

⁹ To perform the mutation calculations, the software utilizes a gender specific rate of mutation for each locus.

4.1.2.4.3.7 If loci should be omitted from the KI calculation because of linkage, select “Yes” in the “Omit?” column next to the locus for each population. Most commonly, D5S818 and vWA are omitted from pedigrees that are not simple paternity or simple reverse paternity. See 4.1.2.2 for additional information to determine which loci should be omitted.

4.1.2.4.3.8 Enter the lab number, Examiner name and/or symbols and date in the corresponding boxes.

4.1.2.4.4 Generating the Report

4.1.2.4.4.1 Navigate to the appropriate report tab. For scenarios in which custom pedigrees were used, the reports will be found on the “Report Cust Ped 1” tab if the manual alternate pedigree setting was set to “No” and the “Report Cust Ped 2” if it was set to “Yes”.

For paternity cases, click on the “Paternity” button at the bottom of the report sheet. The reported combined KI is equivalent to the combined paternity index (PI). Additionally, the probability of paternity calculation and an explanatory statement for the prior probability applied to the calculation will appear. See Figure 11.

Return to KI

To Report Page 2

Print Report

Print This Page Only

Item #	Legal relationship to the TEST individual
	TEST

Combined KI

AFR

Caucasian

SW Hispanic

Probability of Paternity

Probability of paternity was calculated assuming a prior probability of 50%. Prior probability refers to the initial odds of the alleged father's paternity before genetic testing is performed.

Databases: References:

AfAm FSI Genetics 25 (2016) 175-181

Caucasian FSI Genetics 25 (2016) 175-181

SW Hispanic FSI Genetics 25 (2016) 175-181

Allele probability: $1/2N$

Minimum probability for rare alleles: $5/2N$

Theta set to 0. Mutations were not allowed. Silent alleles were not considered.

PATERNITY

Add KI/Pool

Figure 11 – Viewing the “Report” tab for Paternity Analysis

4.1.2.4.4.2 Verify that the displayed information is correct (Lab number, Examiner, date, relationships and profiles). If incorrect information is noted, the user can navigate back to previous tabs to correct the information. The alleles can be corrected in the “Profiles ID” sheet, the pedigree can be corrected in the “Pedigree” or “Custom Pedigree” sheet, and the sample identifiers can be corrected in the “KI” sheet. The user can navigate to these sheets either by selecting the corresponding tabs at the bottom of the workbook or by using the “Return to KI” button and then selecting the “To Profiles ID” or “To Pedigree” buttons. Once changes are made, follow the procedure from the corresponding step forward so that the correct calculation is obtained.

4.1.2.4.4.3 All profiles used to generate the calculations are available on the second page of the report. This second page can be accessed by clicking on “To Report Page 2” or navigating to the tab with the same name.

4.1.2.4.4.4 Click on the “Print Report” button to print both pages of the report.

4.1.2.4.4.5 Once the KIn CALc report is generated, the user may go to the KI tab to calculate the statistic(s) for additional population(s), as appropriate.

4.1.2.4.4.6 To perform calculations for another pedigree (e.g., a different case), click on the “Return to KI” button from the report view. On the KI calculator screen, click on the “Clear Data” button. On the Kit Conversion screen, click on “Clear Data.” The software is now ready for a new analysis.

4.1.2.4.4.7 Once all reports have been printed, the user can proceed to close the software **without saving changes.**

5 Interpretation of Kinship Analysis Results

The weight of the statistical value varies depending on the available reference samples and their relationship to the Test. If first degree relatives are available (e.g., parents, offspring, full-siblings), the probability of obtaining a high KI is increased as compared to when second degree relatives (e.g., half-siblings, grandparents, uncle/aunt) or third degree relatives (e.g., cousins) are used for comparison. Generally, the highest KI's are expected for the pedigrees listed in Section A of Table 1.¹⁰ The pedigrees listed under Sections B and C are less informative.

Whenever possible, the contributor should be directed to collect samples that have a potential to give the greatest KI. Testing additional relatives, if available, can further refine the relatedness of the individuals in question, especially in instances where the combined KI is low.¹¹

¹⁰ Table 1 was adapted from Ge et. al., 2011.

¹¹ Lineage markers (i.e., mitochondrial DNA or Y-STR typing) can also aid in establishing relatedness.

Available known references
SECTION A
3 children + spouse
4 children
Both parents
2 spouses + 2 children (1 each)
2 children + spouse
3 children
1 parent + 3 full-siblings
1 child + 1 parent + spouse
Spouse + 1 child + 1 child w/2 nd spouse
4 full siblings
1 full-sibling + 1 child + spouse
1 parent + 2 full-siblings
3 full-siblings
1 child + 1 parent
2 children
1 full sibling + 1 child
1 full sibling + 1 parent
1 child + spouse
2 full siblings
1 half sibling + 1 parent (not the parent of the half sibling)
1 uncle + 1 parent (they are not related)
1 grandchildren + 1 child (they are uncle/nephew)
1 parent OR 1 child
SECTION B
1 half sibling + 1 full sibling
1 full sibling
SECTION C
2 uncles (they are not related)
2 grandchildren (who are cousins)
2 half siblings (2 halvesibs are also halvesibs)
2 half siblings (2 halvesibs are fullsibs)
2 grandchildren (who are fullsibs)
2 uncles (who are fullsibs)
1 grandparent OR 1 grandchild
1 uncle OR 1 nephew
1 half sibling
2 cousins (they are also cousins)
2 cousins (they are full siblings)
1 cousin

Table 1

5.1 Reporting Procedures

5.1.1 A statement should be included to define the relationships of the submitted reference samples to each other and, if appropriate, the named missing person. This information should be reported generally as follows:

Redacted is identified by the incoming communication from the contributor as the biological mother of the missing person,

are identified by the incoming communication from the contributor as the biological mother and brother, respectively, of the missing person,
.”

is identified by the incoming communication from the contributor as the biological mother of .”

5.1.2 If Y-STR or mitochondrial DNA testing has excluded the putative relationship, no kinship analysis will be conducted for the specimens in question. A statement explaining the reason no comparisons were conducted will be included generally as follows:

“The request for the nuclear DNA comparison of item 1 to Redacted was not performed due to the exclusionary results of the Y-STR comparisons.”

“The request for the nuclear DNA comparison of item 1 to specimen was not performed due to the exclusionary results of the mitochondrial DNA comparison provided in the FBI Laboratory report dated December 1, 2000.”

“The request for the nuclear DNA comparison of item 3 to was not performed due to the exclusionary results of the mitochondrial DNA comparison provided in the State Police Laboratory report dated December 1, 2000, for .”

5.1.3 Unrelated Specimens: Exclusions

5.1.3.1 The comparison of individual DNA profiles in relationships that have an expected pattern of inheritance of alleles (e.g., parent-offspring) may be deemed an exclusion when the expected pattern of allele transmission is not observed. The ability to exclude is limited to those instances in which the STR typing results of the References are able to define or partially define the potential alleles for the Test at a given locus(i). Generally, the ability to exclude is limited to: (1) parent-offspring, (2) multiple siblings, (3) both maternal grandparents, or (4) both paternal grandparents. Combinations of these relatives with additional first and second degree relatives may also allow the Examiner to exclude. Other relative scenarios may also result in an exclusion, but they require numerous relatives (e.g., multiple maternal or paternal aunts/uncles).

5.1.3.2 An exclusion may be declared when the DNA typing results do not fit the proposed

relationships at four or more corresponding loci. At each locus that violates the pedigree, the software will give a KI of 0 or less for that locus and will shade the denominator box(es) in red or the result box(es) in purple. This shading should prompt the Examiner to determine if a mutation approach should be employed. If only one, two, or three loci are shaded, the Examiner should apply the mutation approach. An exclusionary conclusion should be reported generally as follows:

“Based on the STR typing results, item 1 is excluded as being from a biological offspring of [red box]; therefore, item 1 could not have originated from [red box].”

“Based on the STR typing results, [red box] is excluded as being the biological father of [red box].”

“Based on the STR typing results, item 1 is excluded as a being from the biological father of [red box]. Therefore, item 1 could not have originated from [red box].”

“Based on the STR typing results, item 1 is excluded as being from a sister of [red box]; therefore, item 1 could not have originated from [red box].”

“Based on the STR typing results, item 1 is excluded as a being from a potential grandchild of [red box] and [red box]. Therefore, item 1 could not have originated from [red box].”

5.1.4 Inconclusive Results

5.1.4.1 A DNA profile is inconclusive for statistical purposes if, at all loci for which results were obtained, only one allele is detected and it is below the stochastic threshold (ST).¹²

5.1.4.2 If a DNA profile is inconclusive at some loci, but is conclusive at one or more loci, the conclusive loci may be used for statistical calculations as described. All loci should be considered when assessing relatedness.

5.1.4.3 Any profile with 3 or fewer inconclusive loci, and no conclusive loci, is unsuitable for comparisons because 4 loci are needed to exclude from a pedigree.

5.1.4.4 If enough pedigree information is available and at least 4 loci exclude relatedness, an inconclusive DNA profile may be used for an exclusion. Pedigrees that may allow for exclusions include both parents, a spouse and offspring, or multiple siblings.

5.1.4.5 If the pedigree information is too limited to exclude relatedness (e.g., only a single

¹² Refer to the appropriate interpretation SOP of the *DNA Procedures Manual* for determination of inconclusive profiles.

relative is available for comparison), an inconclusive DNA profile cannot be used for comparisons.

5.1.4.6 A statement that describes the inconclusive result and the limitations of the comparisons should be included generally as follows:

“The STR typing results obtained for item 1 are not suitable to determine relatedness; however, they may be utilized for exclusionary purposes. Based on the STR typing results, item 1 is excluded as being from an offspring of _____; therefore, item 1 could not have originated from _____.”*

“The STR typing results obtained for item 1 are not suitable to determine relatedness; however, they may be utilized for exclusionary purposes. Based on the STR typing results, no comparison information for item 1 can be provided for _____.”*

“The limited STR typing results obtained for item 1 are not suitable to determine relatedness. Therefore, no comparisons were made to _____.”*

Each with the following explanatory endnote:

*“*STR typing results are deemed not suitable to determine relatedness (i.e., inconclusive) when the potential exists that not all of the genetic information in a biological sample has been detected. For STR typing results to be used to determine relatedness, sufficient DNA quality and/or quantity is necessary, and will depend on the relative samples submitted.”*

5.1.5 Reporting Combined Kinship Indices

5.1.5.1 Combined kinship indices¹³ are calculated using four general United States population groups (i.e., African-American, Caucasian, Southwestern Hispanic, and Southeastern Hispanic). Additional kinship indices may be reported for specimens that potentially originated from Native American populations (i.e., Apache, Navajo, and Minnesota Native American) or Caribbean populations (i.e., Trinidadian).¹⁴ The lowest combined KI should be reported generally as follows:

“Based on the STR typing results and the comparisons of item 1 to _____, the combined kinship index is approximately 150 million.”*

¹³ All kinship indices are reported rounded to two significant figures as provided by KIn CALc, or, if between 1 and 10, truncated to 1 significant figure. For example, 13,423 is rounded to 13,000; 54,784,652 is rounded to 55,000,000, or 55 million; 3,751,768,135 is rounded to 3,800,000,000, or 3.8 billion.

¹⁴ The use of Native American or Caribbean population databases is generally based on the geographic location of the requesting agency or the population associated with the pedigree. With TL approval, other population databases may be similarly used.

with the following associated explanatory endnote:

*“*Not all loci at which amplification is attempted will yield interpretable results; a statistical estimate (combined kinship index) has been based on loci with conclusive typing results. Calculations were performed using the African American, Caucasian, Southeastern Hispanic, and Southwestern Hispanic populations. The lowest combined kinship index calculated from these populations is reported.”¹⁵*

5.1.5.2 The combined paternity index (PI) is a specialized combined kinship index. It is calculated for the four general United States population groups (i.e., African-American, Caucasian, Southwestern Hispanic, and Southeastern Hispanic). Additional kinship indices may be reported for specimens that potentially originated from Native American populations (i.e., Apache, Navajo, and Minnesota Native American) or Caribbean populations (i.e., Trinidadian). The lowest combined PI and the probability of paternity should be reported generally as follows (where **Redacted** is the alleged father, **Redacted** is the known mother, and **Redacted** is the child):

“Based on the STR typing results and the comparisons of **Redacted**, the combined paternity index is approximately 23,000 **”*

with the following explanatory endnotes, which include the probability of paternity:

*“*Not all loci at which amplification is attempted will yield interpretable results; a statistical estimate (combined paternity index) has been based on loci with conclusive typing results. Calculations were performed using the African American, Caucasian, Southeastern Hispanic, and Southwestern Hispanic populations. The lowest combined paternity index calculated from these populations is reported.”*

***The corresponding probability of paternity is 99.995%. The probability of paternity is expressed as a percentage that incorporates the combined paternity index and a 50% prior probability that the tested man is the biological father of the child.”*

5.1.5.3 Section A Pedigrees

Pedigrees with the most value for kinship analysis consist of individual or multiple first degree relatives,¹⁶ or a first degree relative along with a second degree relative.¹⁷ These pedigrees, which also include pedigrees for paternity analysis, are captured in Section A of Table 1.

5.1.5.3.1 If the reported combined kinship index is greater than one, an additional clarifying

¹⁵ This endnote is only appropriate for analyses where the alternate hypothesis is unrelatedness. This endnote should be modified as appropriate to address relationships assessed by multi-category pairing or custom pedigrees.

¹⁶ A first degree relative is a direct descendant, predecessor, or full sibling of the person in question, i.e., a parent, child, or full sibling. Additionally, though spouse is not a biological relative, it is important to the evaluation if children are available for typing.

¹⁷ A second degree relative is a direct descendant, predecessor, or full sibling of a first order relative, i.e., a grandparent, grandchild, or full sibling of the parent of the person in question.

statement will be added generally as follows:

“Therefore, the profile obtained from item 1 is approximately 150 million times more likely if item 1 is from the child of Redacted than if item 1 is from someone unrelated to these individuals.”

“Therefore, the profile obtained from item 1 is approximately 150 million times more likely if item 1 is from the sister of Redacted than if item 1 is from someone unrelated to these individuals.”

“The profile for Redacted [child] is approximately 280 million times more likely if Redacted [known parent] and Redacted [alleged parent] are the parents than if Redacted [known parent] and an unrelated randomly selected man are the parents.”

5.1.5.3.2 A statement summarizing the strength of the evidence should follow. See Table 2 for a summary of reporting language for Section A pedigrees.

Combined KI of	Reported as
100,000 or greater	Strong evidence
1,000 to 99,999	Evidence
100 to 999	Cannot be excluded
1 to 99	Insufficient support to conclude relatedness
0 to 1	Unlikely

Table 2 - Reporting Language for Section A Pedigrees

5.1.5.3.3 Report Wording Examples for Section A Pedigrees

The qualitative equivalent of the combined KI or PI is based on the magnitude of the reported LR. These conclusions should be reported generally as follows:

“These results provide strong evidence that item 1 originated from Redacted .”*

“These results provide strong evidence that Redacted is the biological father of Redacted .”*

“These results provide evidence that item 1 originated from Redacted .”*

“These results provide evidence that Redacted is the biological father of Redacted .”*

“Therefore, item 1 cannot be excluded as having originated from Redacted .”*

“Therefore, Redacted cannot be excluded as the biological father of Redacted .”*

“These results provide insufficient support to conclude that item 1 originated from Redacted.”*

“These results provide insufficient support to conclude that is the biological father of.”*

“Therefore, it is unlikely that item 1 originated from.”*

“Therefore, it is unlikely that is the biological father of.”*

with the following associated explanatory endnote:

*“*These combined kinship/paternity index (KI/PI) ranges provide the following support for the conclusion:*

<u>KI/PI</u>	<u>Qualitative Equivalent</u>
$\geq 100,000$	strong evidence of relatedness
1,000 to 99,999	evidence of relatedness
100 to 999	cannot be excluded as related
1 to 99	insufficient support to conclude relatedness
0 to 1	unlikely to be related”

5.1.5.4 Section B Pedigrees

Pedigrees consisting of a single full sibling or a full sibling and a half sibling are sometimes useful in establishing kinship. However, comparison of true relatives in this category will occasionally result in KIs between zero and one. Additionally, there is a potential for fortuitous associations. Therefore, the report should include a request for additional, informative, relative samples from the contributor. These two pedigree types are captured in Section B of Table 1.

5.1.5.4.1 If the reported combined kinship index is greater than one, an additional clarifying statement should generally as follows:

“Therefore, the profile obtained from item 1 is approximately 4,500 times more likely if item 1 is from the brother of than if item 1 is from someone unrelated to this individual.”

“Therefore, the profile obtained from item 1 is approximately 4,500 times more likely if item 1 is from the brother and half-brother, respectively, of than if item 1 is from someone unrelated to these individuals.”

5.1.5.4.2 A statement summarizing the strength of the evidence should follow. See Table 3 for a summary of reporting language for Section B pedigrees.

Combined KI of	Reported as
100,000 or greater	Strong evidence
1,000 to 99,999	Evidence
100 to 999	Cannot be excluded
0 to 99	Insufficient support to conclude relatedness

Table 3 - Reporting Language for Section B Pedigrees

5.1.5.4.3 Report Wording Examples for Section B Pedigrees

The qualitative equivalent of the combined KI is based on the magnitude of the reported LR. These conclusions should be reported generally as follows:

“These results provide strong evidence that item 1 originated from Redacted ; however, further testing from at least one additional relative (preferably a parent, child, or full sibling) is recommended to support this conclusion.”*

“These results provide evidence that item 1 originated from Redacted ; however, further testing from at least one additional relative (preferably a parent, child, or full sibling) is recommended to support this conclusion.”*

“Therefore, item 1 cannot be excluded as having originated from Redacted ; however, further testing from at least one additional relative (preferably a parent, child, or full sibling) is recommended to support this conclusion.”*

“These results provide insufficient support to conclude that item 1 originated from Redacted ; however, further testing from at least one additional relative (preferably a parent, child, or full sibling) is recommended.”*

with the following associated explanatory endnote:

*“*These combined kinship index (KI) ranges provide the following support for the conclusion:*

<u>KI</u>	<u>Qualitative Equivalent</u>
$\geq 100,000$	<i>strong evidence of relatedness</i>
1,000 to 99,999	<i>evidence of relatedness</i>
100 to 999	<i>cannot be excluded as related</i>
0 to 99	<i>insufficient support to conclude relatedness”</i>

5.1.5.5 Section C Pedigrees

Pedigrees consisting of one or two second degree relatives¹⁸ or third degree relatives¹⁹ are at times useful in establishing kinship. However, comparison of true relatives in this category may result in low KIs and KIs less than one. Additionally, as with Section B pedigrees, there is a potential for fortuitous associations. These pedigree types are captured in Section C of Table 1. Because of the limitations in the analysis of these pedigrees, there are only two categories of reporting. Additionally, the report should include a request for additional, informative, relative samples from the contributor.

5.1.5.5.1 A statement explaining the limited value of the relationships should be added to the paragraph identifying the relatives submitted for analysis, generally as follows:

Redacted is identified by the incoming communication from the contributor as the maternal uncle of the missing person, Redacted. It is noted that comparisons to maternal uncles have limited value because second degree relatives are expected to share limited genetic information by descent."

Redacted are identified by the incoming communication from the contributor as the cousins of the missing person, Redacted. It is noted that comparisons to cousins have limited value because third degree relatives are expected to share limited genetic information by descent."

5.1.5.5.2 A statement summarizing the strength of the evidence should follow. See Table 4 for a summary of reporting language for Section C pedigrees.

Combined KI of	Reported as
100 or greater	Cannot be excluded
0 to 99	Insufficient support to conclude relatedness

Table 4 - Reporting Language for Section C Pedigrees

5.1.5.5.3 If the reported combined kinship index is greater than one, an additional clarifying statement will be added generally as follows:

"The profile obtained from item 1 is at least 560 times more likely if item 1 is from the cousin of Redacted than if item 1 is from someone unrelated to this individual."

"The profile obtained from item 1 is at least 560 times more likely if item 1 is from the grandparent of Redacted and Redacted than if item 1 is from someone unrelated to these individuals."

¹⁸ A second degree relative is a direct descendant, predecessor, or full sibling of a first degree relative, i.e., a grandparent, grandchild, or full sibling of the parent of the person in question.

¹⁹ A third degree relative is a direct descendant, predecessor, or full sibling of a second degree relative, e.g., a cousin, great-uncle, of the person in question.

5.1.5.5.4 Report Wording Examples for Section C Pedigrees

The qualitative equivalent of the combined KI is based on the magnitude of the reported LR. A request for additional relative samples from the contributor to further refine the relationships is also included. These conclusions should be reported generally as follows:

“Therefore, item 1 cannot be excluded as having originated from Redacted ; however, further testing from at least one additional relative (preferably a parent, child, or full sibling) is necessary to support this conclusion.”*

“These results provide insufficient support to conclude that item 1 originated from Redacted ; however, further testing from at least one additional relative (preferably a parent, child, or full sibling) is necessary.”*

with the following associated explanatory endnote:

*“*These combined kinship index (KI) ranges provide the following support for the conclusion:*

<i>KI</i>	<i>Qualitative Equivalent</i>
<i>≥ 100</i>	<i>cannot be excluded as related</i>
<i>0 to 99</i>	<i>insufficient support to conclude relatedness</i>

However, because of the relationships of the individuals whose samples were provided, this comparison has limited value in drawing conclusions with respect to relatedness.”

5.1.6 Haplotype Results

Autosomal kinship associations can be supported by haplotype results (i.e., Y-STR and/or mitochondrial DNA results).

5.1.6.1 With the approval of the TL, autosomal and haplotype statistics (e.g., Y-STR and/or mitochondrial DNA results) may be combined.²⁰

5.1.6.2 If inclusionary mitochondrial DNA results have been reported, a statement that additional results are available may be included:

“It is noted that the results of the mitochondrial DNA examinations were the subject of a separate report. Please refer to the FBI Laboratory report Redacted dated January 1, 2000 for the results of the mitochondrial DNA examinations.”

Additionally, in the Remarks section of the report, the contributor can be directed to phone the Mitochondrial DNA Examiner to obtain further information.

²⁰ Ge et. al. 2010.

6 Limitations

6.1 It is not possible to anticipate the nature of all potential biological relationships.

6.2 The strength of the combined kinship index is dependent on the References submitted by the contributor. Based on the samples provided and the results obtained, some pedigrees have limited value in determining relatedness.

- When limited genotypes are obtained, a KI may be obtained that has limited value to conclude relatedness even when true relatives are analyzed.
- Section B and C pedigrees have the potential to result in a KI that supports unrelatedness even when true relatives are analyzed. Additionally, there is a potential for fortuitous associations with Section B and C pedigrees.

6.3 KIn CALc software is not validated and will not be used to calculate the KI for relationships beyond first cousins.

6.4 The KI cannot be used to predict the population from which the source of an evidence sample originated.

7 Calculations

See Appendix A.

8 Standards and Controls

Not applicable.

9 Sampling

Not applicable.

10 Measurement Uncertainty

Not applicable.

11 Safety

Not applicable.

12 References

- Ayres KL. Relatedness testing in subdivided populations, *Forensic Science International* (2000) 114:107-115.
- AABB (American Association of Blood Banks). 2008. *Annual Report Summary for Testing in 2008*. Relationship Testing Program Unit.
- Buckleton J, Clayton T, Triggs C. Parentage Testing. *Forensic DNA Evidence Interpretation*. CRC Press (2005), p. 342-384.
- Budowle B, Moretti TR, Baumstark AL, Defenbaugh DA, Keyes KM. Population data on the thirteen CODIS core short tandem repeat loci in African Americans, U. S. Caucasians, Hispanics, Bahamians, Jamaicans, and Trinidadians, *Journal of Forensic Sciences* (1999) 44(6):1277-1286.
- Budowle B, Chakraborty R. Population variations at the CODIS core short tandem loci in Europeans, *Legal Medicine* (2001) 3:29-33.
- Budowle B, Collins PJ, Dimsoski P, Ganong CK, Hennessy LK, Leibelt CS, Rao-Coticone S, Shadravan F, Reeder DJ. Population Data on the STR Loci D2S1338 and D19S433, *Forensic Science Communications* (2001) 3(3).
- Budowle B, Defenbaugh DA, Keyes KM. Genetic variation at nine short tandem repeat loci in Chamorros and Filipinos from Guam, *Legal Medicine* (2001) 2:26-30.
- Budowle B, Shea B, Niezgoda S, Chakraborty R. CODIS STR loci data from 41 sample populations, *Journal of Forensic Sciences* (2001) 46(3):453-489.
- Budowle B. Population studies on the 17 STR loci routinely used in forensic analysis. In: *International Congress Series*, 1028 (2002).
- Burri H, Sulzer A, Voegeli P and Kratzer A. Accounting for linkage between the STR loci D5S818/CSF1PO and vWA/D12S391 in kinship analyses: Impact on likelihood ratio values. *FSI Genetics* (2015) e387-389
- Chakraborty R. Sample size requirements for addressing the population genetic issues of forensic use of DNA typing, *Human Biology* (1992) 64(2):141-159.
- DNA Advisory Board. Statistical and Population Genetics Issues Affecting the Evaluation of the Frequency of Occurrence of DNA Profiles Calculated From Pertinent Population Database(s), *Forensic Science Communications* (2000) July 2(3).
- Elston RC, Stewart J. A general model for the genetic analysis of pedigree data, *Human Heredity* (1971) 21:523-542.

Federal Bureau of Investigation, *Quality Assurance Standards for Forensic DNA Testing Laboratories*, 2011.

Ge J, Eisenberg A, Budowle B. Developing criteria and data to determine best options for expanding the core CODIS loci, *Investigative Genetics* (2012)3:1.

Ge J, Budowle B, Chakraborty R. DNA identification by pedigree likelihood ratio accommodating population substructure and mutations, *Investigative Genetics* (2010) 1:8.

Ge J, Budowle B, Chakraborty R. Choosing relatives for DNA identification of missing persons, *Journal of Forensic Sciences* (2011) 58(S1):S23-S28.

Gill P, Phillips C, McGovern C, Bright JA and Buckleton J. An evaluation of potential allelic association between the STRs vWA and D12S391: Implications in criminal casework and applications to short pedigrees. *FSI Genetics* (2012) 6:477-486

Lewis O'Connor K, Hill CR, Vallone PM and Butler JM. Linkage disequilibrium analysis of D12S391 and vWA in U.S. population and paternity samples. *FSI Genetics* (2011) 5:538-540.

Li HX, Tong DY, Lu HL, Ou XL, Chen WJ, Zhang YM, Liu SJ, Chen Y, Sun HY. Mutation analysis of 24 autosomal STR loci using in paternity testing, *FSI Genetics* (2011)3:e159-e160

Lu D, Liu Q, Wu W and Zhao H. Mutation analysis of 24 short tandem repeats in Chinese Han population. *Int J Legal Med* (2012) 126:331-335.

Moretti TR, Moreno LI, Smerick JB, Pignone ML, Hizon R, Buckleton JS, Bright JA and Onorato AJ. Population data on the expanded CODIS core STR loci for eleven populations of significance for forensic DNA analyses in the United States, *FSI Genetics* (2016) 25:175-181.

National Research Council. *The Evaluation of Forensic DNA Evidence*, Washington, D.C.: National Academy Press, 1996.

DNA Procedures Manual.

O'Connor, KL. Interpretation of DNA Typing Results for Kinship Analysis. Proceedings from USCIS Working Group on DNA Policy; 2011 Jan 25; Washington, DC.

Ruitberg CM, Reeder DJ, Butler JM. STR BASE: A short tandem repeat DNA database for the human identity testing community, *Nucleic Acid Research* (2001) 29(1).

Wenk RE, Traver M, Chiafari FA. Determination of sibship in any two persons, *Transfusion* (1996) 36:259-262.

Rev. #	Issue Date	History
2	11/05/13	<p>Changed “LR” to “KI” for consistency throughout.</p> <p>Changed “smallest” to “lowest” throughout.</p> <p>Updated all figures to account for the new software version.</p> <p>2: Added clarifying wording and added reference to Appendix A.</p> <p>3: Updated KIn CALc Software Version.</p> <p>7.4: Added statement regarding exclusions.</p> <p>7.4.1.1: Added “generally” as not all pedigrees fit this model.</p> <p>7.4.1.2.1.1: Reworded for clarity.</p> <p>Footnote 1: Added amelogenin results are not included in KI.</p> <p>7.4.1.2.2: Added the option to use the Pedigree or the Custom Pedigree tab. The original sections are renumbered for the Pedigree Tab (7.4.1.2.2.1).</p> <p>7.4.1.2.2.1: Added “for Standard Pedigrees”.</p> <p>7.4.1.2.2.1.5, 7.4.1.2.2.1.5.1: New section Pedigree Tab for Non-Standard Pedigrees, including Figure 6.</p> <p>7.4.1.2.2.2: New section Custom Pedigree Tab for Non-Standard Pedigrees, including Figures 7 and 8. Renumbered remaining figures.</p> <p>7.4.1.2.3: Removed references to specific databases.</p> <p>7.4.1.2.3.1, 7.4.1.2.4.1, 7.4.1.2.4.2: Reworded to allow for the Custom Pedigree.</p> <p>7.4.1.2.3.2: Changed “initial screen” to “Profile ID tab”.</p> <p>7.4.1.2.3.3: Revised for clarity. Revised footnote 3 for clarity. Added footnote 4.</p> <p>7.4.1.2.4: Added information regarding which report tab to use.</p> <p>Removed “Report Tab” figure. Reorganized steps within this section for clarity.</p> <p>7.4.1.2.4.4: Reworded for clarity and removed last sentence.</p> <p>7.5: Removed first paragraph as it is repeated from the background section 2. Last paragraph is moved from section 7.6.6 and revised for clarity. Added footnote 9.</p> <p>7.6.2: Revised report wording for clarity.</p> <p>7.6.3: Removed “autosomal” describing Y-STRs.</p> <p>7.6.4: Revised for clarity.</p> <p>7.6.4.1, 7.6.4.2: Moved footnotes into the body of the document.</p> <p>Revised the report wording for clarity.</p> <p>7.6.5.1: Revised the report wording for clarity.</p> <p>7.6.6: Moved first paragraph to 7.5, deleted second paragraph, and renumbered remaining sections.</p> <p>7.6.6.1: Revised footnote 14 for clarity. Removed footnote referencing NRC 1996. Added footnote 15.</p> <p>7.6.6.2: Added option to calculate other PIs. Divided into 7.6.6.2.1, 7.6.6.2.2, and 7.6.6.2.3.</p> <p>7.6.6.2.2: Revised for clarity.</p> <p>7.6.6.2.3: Added last sentence and footnote. Added footnote 16.</p> <p>Revised report and endnote wording for clarity.</p>

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- 7.6.6.3.1: Changed wording in paternity example to more accurately represent the KI.
 - 7.6.6.3.2.1: Revised report wording.
 - 7.6.6.3.2.2: Revised paternity wording for clarity. Changed 99,000 to 99,999 in the endnote wording.
 - 7.6.6.3.2.3, 7.6.6.3.2.4, 7.6.6.3.2.5: Revised endnote wording for clarity. Revised paternity wording for clarity.
 - 7.6.6.4: Changed “will” to “should” for requesting additional samples.
 - 7.6.6.4.2.1, 7.6.6.4.2.3, 7.6.6.4.2.4: Revised endnote wording for clarity.
 - 7.6.6.5: Revised for clarity; changed “will” to “should” for requesting additional samples.
 - 7.6.6.5.3: Changed 100 to 1 for adding an additional clarifying statement.
 - 7.6.6.5.4: Revised body and endnote wording for clarity.
 - 7.6.6.5.5: Changed one to zero and revised endnote wording for clarity.
 - 7.6.7: Added that KIs can be supported by mtDNA and/or Y-STR.
 - 7.6.7.1: Added the option to combine autosomal KIs with haplotype results with TL approval.
 - 7.6.7.2: Revised for clarity.
 - 8: Moved section to Appendix A and renumbered remaining sections.
 - 8.2: Expanded description of limitations.
 - 8.3: Revised for clarity.
 - 8.4: Removed.
 - Appendix A: Moved from previous section 8 with the following modifications:
 - 1.1: Added Table 8 to the listing.
 - 1.5: Added “Combined” to KI; removed the example.
 - 1.6: Removed the example.
- 3 07/27/17 Updated entire document to reflect updated software version and the ability to enter GF loci, including figures.
Removed nDNAU throughout.
Moved N/A sections to the end and renumbered.
- 4.1.2.1: changed theta to 0.01 for most populations and 0.03 for Native American populations.
 - 4.1.2.2: added Linkage section and guidance.
 - 4.1.2.3: added guidance for AT settings for kinship calculations.
 - 4.1.2.4.2: limited the use of the custom pedigree to two scenarios; alternate scenarios require TL approval.
 - 5: removed columns from Table 1, removed Table 2 entirely.
 - 5.1: updated reporting procedures to make similar to current reporting practices for GF interpretation of direct comparisons, including replacing “specimen” with “item”, reporting only the lowest KI/PI, including the verbal scale table in the report .
 - 5.1.3.2: require 4 loci to determine an exclusion.
 - 5.1.4: provide additional guidance for inconclusive profiles, including when a profile is unsuitable for comparisons.

6: Relocated statement regarding what analysis will be conducted from the Limitations to the Background.

6.3, 6.4: added Limitations.

12: Added multiple references.

Appendix A, 1.1: removed tables for hand calculations and cited the appropriate source.

Appendix A, 1.3: update to clarify.

Appendix A, 3.1.3: updated Table to include GF loci and cited appropriate sources.

Approval

Redacted - Signatures on File

Appendix A: Calculations

1 Calculation of Kinship Indices for Single Relative and Parent Samples

1.1 The formulae for the calculation of likelihood ratios that incorporate a coancestry coefficient on a single locus basis for situations involving parents and/or offspring; paternity; reverse paternity; full-siblings; uncle-nephew, half-siblings and grandparent-grandchild; and cousins are found in Ayres, 2000.²¹

1.2 Single-locus KIs are multiplied to obtain the multi-locus kinship index that represents the likelihood of biological relatedness, as follows:

$$\text{Combined KI} = \text{KI}_{\text{LOCUS1}} \times \text{KI}_{\text{LOCUS2}} \times \dots \times \text{KI}_{\text{LOCUS}(n)}$$

1.3 The probability of paternity is calculated using the following formula:

$$= \frac{(\text{CPI} \times \text{Pr})}{[\text{CPI} \times \text{Pr} + (1 - \text{Pr})]}$$

Where CPI = combined PI and Pr = prior probability

The Pr is set to 0.5, which simplifies the probability of paternity to:

$$= \frac{\text{CPI}}{(\text{CPI} + 1)}$$

KIn CALc calculates the probability of paternity and reports the percentage truncated to six significant digits. The examiner will report the probability of paternity, expressed as a percentage, rounded to five significant digits. However, any value greater than 99.999% will be reported as greater than 99.999% and will not be rounded to 100%.

2 Calculation of Kinship Indices when Multiple Relative Samples are Available

2.1 Elston-Stewart

For cases in which multiple alleged relatives (other than parents) are available, the number of iterations of possible genotype combinations becomes too large to be able to calculate by hand. In these situations the software is designed to use the Elston-Stewart algorithm.²²

$$L = \sum_{G_{\text{founder}}} P(X_{\text{founder}} | G_{\text{founder}}) H(G_{\text{founder}}) P(G_{\text{founder}})$$

The Elston-Stewart algorithm considers the probability of founder²³ genotypes and the probability of offspring given the parents.

²¹ Ayres KL. Relatedness testing in subdivided populations, *Forensic Science International* (2000) 114:107-115.

²² This algorithm is described in Human Heredity 21:523-542 (1971).

²³ A founder is a person in the pedigree for which no antecedent genetic information is available.

3 Calculations Involving Mutations

3.1 ‘Ayers’ Mutations Approach

3.1.1 The probability of a maternal allele not mutating is $1 - \mu_{\text{maternal}}$; the probability of a paternal allele not mutating is $1 - \mu_{\text{paternal}}$.

3.1.2 The probability of a maternal allele mutating is $\frac{1}{2} * \mu_{\text{maternal}} * (1/10)^{s-1}$. The probability of a paternal allele mutating is $\frac{1}{2} * \mu_{\text{paternal}} * (1/10)^{s-1}$. In both cases, $\frac{1}{2}$ is the probability of an allele mutating, μ is the mutation rate for the locus in question and s is the number of steps the allele underwent before becoming the “mutated” allele (e.g., if an 11 mutated to 13, $s = 2$).

3.1.3 The mutation rates (μ) for the different loci are found in Table 5 as reported in the AABB 2008 report and in Lu et.al. Int J Legal Med (2012).

	Maternal μ	Paternal μ
CSF1PO	0.000283	0.002021
D10S1248	0	0.0025
D12S391	0.00032	0.003
D13S317	0.000436	0.001743
D16S539	0.000481	0.001127
D18S51	0.000748	0.00253
D19S433	0.000596	0.000745
D1S1656	0	0.0025
D21S11	0.001295	0.001709
D22S1045	0	0.0025
D2S1338	0.000245	0.001526
D2S441	0	0.0025
D3S1358	0.000211	0.001691
D5S818	0.0003	0.001742
D7S820	0.000073	0.001348
D8S1179	0.000333	0.002031
FGA	0.000522	0.003713
SE33	0.00303	0.00639
TH01	0.000043	0.00007
TPOX	0.000081	0.00013
vWA	0.000494	0.003258

Table 5 – Mutation Rates for Various Loci